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BIOACCUMULATION OF PCB 169 AND ITS HISTOPATHOLOGICAL EFFECTS ON THE INTESTINE TISSUE OF FRESHWATER FISH PUNTIUS TICTO

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ABSTRACT

The present work studies the bioaccumulation of Poly Chlorinated Biphenyl congener No. 169 in the intestine tissue of freshwater fish *Puntius ticto* over a 30 days' chronic exposure, and highlights the gradual histopathological changes occurring in the tissue in this period. PCB 169 was seen to accumulate up to 13.23 µg g-1 wet weight of the tissue accompanied by gradual deterioration of intestinal villi. This work showcases the toxic effects of accumulation of PCB 169 in the intestine tissue of the fish.

KEYWORDS: PCB169, Intestine, *Puntius Ticto*, Bioaccumulation, Histopathology

INTRODUCTION

Water, the life-giver revered over time, and chemically a solvent unsurpassed, shelters ecosystems within it, be it marine, freshwater, flowing, stagnant, subterranean or otherwise. As such, life tends to thrive in it, if it remains unpolluted. However, man, in his haste for development, has been developing a plethora of substances for his own benefit, be it crude oil, lubricants, machine fluids, fertilizers, pesticides and many more. Willingly or accidentally, man releases these substances into the environment, and ultimately they end up in water.

While many such substances might dissolve and be relatively harmless to life, there are others that are not so easily degraded, and tend to persist. These are mostly organic in nature and are termed as POPs (persistent organic pollutants). Presence of various types of pollutants in water and their toxic effects began to raise alarms (Holmes, D. C.et.al; 1967), (Arthur, D.R., 1971).

PCBs are one such class of as many as 209 compounds termed as congeners, with as many structural variations caused by substitution of Hydrogen by Chlorine at different positions around the biphenyl ring. The major use of PCB today is as a dielectric in electrical equipment servicing industries. When PCBs are spilled or PCB equipment is abandoned underground, the PCBs can be expected to be released into the ground water with no possibility of source retrieval. This can result in water pollution for which there may be no solution (Bench, D. W., 2003).

While some of the Congeners are less harmful, some are known to be potent toxicants (Falandysz J. et.al; 1994), accumulating to different extent in the bodies of aquatic organisms, the phenomenon being termed as bioaccumulation. Studies on patterns of bioaccumulation are found in literature (Porte, C. and Albaigés, J. , 1993).

Pollutants have been found to persist in sediment (Kolok, A.S. et.al; 1996), (Eklund, B. et.al; 2010), sediment dwellers (Zeng, E.Y. et.al; 2003), different fish, terrestrial invertebrates such as earthworms (Kinney, C.A. et.al; 2008), birds (Swartz, C.D. et.al; 2003), (Daelemans, F. F. et.al; 1992), mammals (Nakata, H. et.al; 1997) man's food (Eljarrat, E. et.al; 2002), (Cordle, F. et.al; 1982) as well as in man himself (Norén, K. et.al; 1990), (Srogi, K., 2008).

These accumulated pollutants are seen to play a role in affecting metabolism (Stow, C. A. et.al; 1997) and physiological processes by interfering with enzyme pathways, interfering with hormones (Hontela,A. et. al; 1992) and also having deleterious effects on tissues and organs (Kumar, A. and Mathur, R.P., 1995) and behavioural patterns (Couillard, C.M. et.al; 2011), which may ultimately prove to be fatal.

Initial work on their effects was done using commercial mixtures such as Arochlor® (D. J. Hansen et.al; 1971, 1974), and only later, the effects of individual congeners began to be studied (Opperhuizen, A. et.al; 1988), (Johnson, B. L. et.al; 2008), (Frame, G. M., 2009).

Focus of studies was usually first on short term effects to determine sub lethal tolerance of these organisms and determination of safe doses that could be administered to them (Basak, P.K. and Konar, S.K. 1977), (Stalling, D. and Mayer, F. L., 1972). Later, the effects of chronic exposure (Al-Rabai' Ah Hussam et.al; 2005), (Borlakoglu, J.T. and Haegele, K.D., 1991), (David J. Hoffman et.al; 1996) were studied in detail by many workers.

The aquatic organisms that accumulate the pollutant also form a part of the food chain, and when consumed, tend to transfer the load of toxicant to the higher orders of the food chain. If it cannot be degraded here also, it tends to increase in load, the phenomenon being termed as biomagnification. Disruptions in food chains may result in wiping out complete ecosystems, especially fragile ones such as coral reef ecosystems.

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Detection of bioaccumulated pollutants and their effects are recognised as biomarkers (Matson, C.W. et.al; 2009), (Fernandes, D. et.al; 2008).

MATERIALS AND METHODS

In this study, PCB 169 has been chosen due to its known toxic nature. The PCB 169 was of Dr. Ehrenstorfer, Germany, make. The freshwater fish *Puntius ticto*, a minor carp, was chosen as the experimental organism for its small size and ease of handling. Specimens were collected from local freshwater bodies. *Puntius ticto* is invariably netted out with major carps, and though considered as trash fish, it is consumed by man in dried or fried form.

Initially, the fish were acclimatized in dechlorinated tap water, the characteristics of which are given in Table 1.

Determination of acute toxicity is the first step in bioassays. Many workers have used various methods to determine a safe dose using static bioassays (Litchfield, J. T. , and Wilcoxon, F., 1949), (Dillon, T.M. and Burton, W.D.S.,1991). In this study, static bioassays for determination of acute toxicity were carried out according to methods recommended in ISI (1971), APHA Standard Methods (1976),and USEPA (1975, 1978). This method of bioassay has been widely used (John C. Davis and Barbara J. Mason, 1973), (Jung -Suk Lee et al, 2005). Following the standard protocol, LC0, LC100 and LC50 values were calculated, to arrive at a safe dose for further chronic bioassay. Appropriate controls were invariably maintained.

Chronic bioassay was carried out using continuous flow through methods as described in literature (Brenniman ,G. et.al; 1976). 20 fish in healthy condition were introduced in a 20 litre aquarium provided with an outlet at the upper level of water. 20 litres of water containing the safe dose of 20 µg l-1 PCB 169 were introduced into the aquarium every 24 hours by allowing it to flow into the aquarium gradually. Fish were thus continuously exposed to the safe dose of PCB 169 every day for a period of 30 days. Feeding and replacements of fresh solutions were followed as per details reported in literature (Murty, A.S., 1986). A control was maintained in parallel.

Test fish were removed from the flow- through aquarium on every 5th day of exposure, and intestine tissue was removed by dissection. Tissue was preserved in Buoin's fixative for 15-20 days until further processing for histological study. Similarly, intestine tissue was also collected every 10 days, i.e; on the 10th, 20th and 30th day. These were preserved in 4% formaldehyde for study of bioaccumulation.

Tissue for histological study was processed for double staining by Haematoxylin -Eosin technique. For study of bioaccumulation, the tissue was thoroughly washed, blotted and wet weight of the tissue was carefully noted. Tissue extract was prepared by maceration in dichloromethane, and repeatedly passed through glass columns packed with filter aid Celite 545 for tissue clean-up. The eluate was allowed to concentrate by allowing dichloromethane to evaporate and stored in tightly stoppered glass tubes.

Analysis of the eluate for estimating bioaccumulation of PCB 169 was carried out using GC-MS of Thermo Fisher make. There are references in literature for use of GC-MS for this purpose (Ballschmiter, K.W. et. al; 1992), (Baumer, A. et.al; 2020). The instrument was calibrated for analysis of PCB 169. Chromatographic conditions were set, and calibration curve and mass spectra were obtained as shown in Fig.1 to Fig.3.

OBSERVATIONS

The bioaccumulation of PCB 169 in the intestine tissue in µg g-1 wet weight was noted on the basis of the chromatograms obtained on analysis using GC-MS. Chromatogram for 10th day of sample is shown in Fig. 4.

The bioaccumulation observed has been tabulated in Table 2. The increasing trend observed during the complete exposure period has been shown graphically in Fig.5.

The histopathological effects were found consistent with and corresponding to the bioaccumulation.

At 10 days' exposure, the intestine showed degeneration of mucosa along with its separation from the underlying tissue. Accumulation of dense granular matter was also observed in the submucosal tissue (Fig.6).

At 20 days' exposure, the tissue showed a distinct flattening of the villi (Fig.7).

The intestinal tissue at 30 days' exposure showed a further degeneration with flattening and further separation of mucosa from the underlying layers (Fig.8).

RESULTS AND DISCUSSION

Bioaccumulation of PCB 169 could be seen to increase gradually over the exposure period with distinct increasing trends as can be seen from Fig. 5. A further exposure would therefore result in further bioaccumulation. Initially, the bioaccumulation was 6.89 µg g-1 of wet weight and went on to increase to 13.23 µg g-1 of wet weight of the intestine. The physical manifestation is evident from the degenerative changes in the intestinal layer observed and shown in Fig. 6 to Fig.8.

CONCLUSION

Degenerative changes seen are irreversible and would ultimately prove fatal to the fish. Moreover, if the fish were consumed in the meantime by a higher consumer, the load of PCB 169 would most likely be transferred to the consumer and would biomagnify, given repeated consumption of fish carrying the toxicant. Protocols for measurement of the toxicant in waters therefore require to be in place and to be implemented regularly to avoid any deleterious effects on the aquatic organisms and their consumers.

FIGURES AND TABLES

Fig .1: Chromatographic Conditions for PCB Analysis

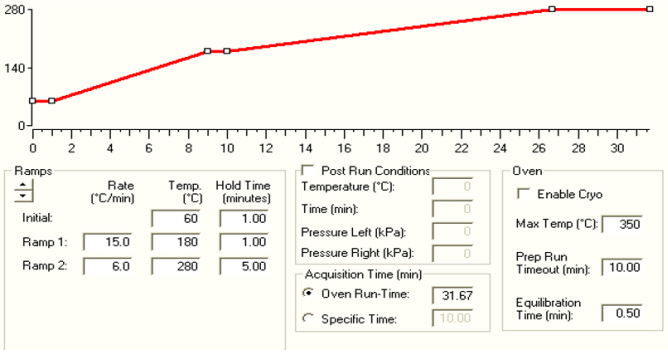

Fig. 2: Mass spectra of PCB-169 (3, 3', 4, 4', 5, 5'-Hexachlorobiphenyl)

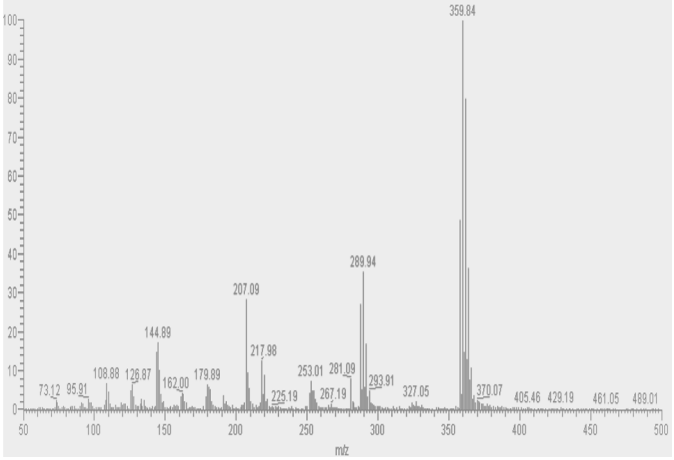

Fig. 3: Standard Chromatogram of PCB-169 (3, 3', 4, 4', 5, 5'-Hexachlorobiphenyl)

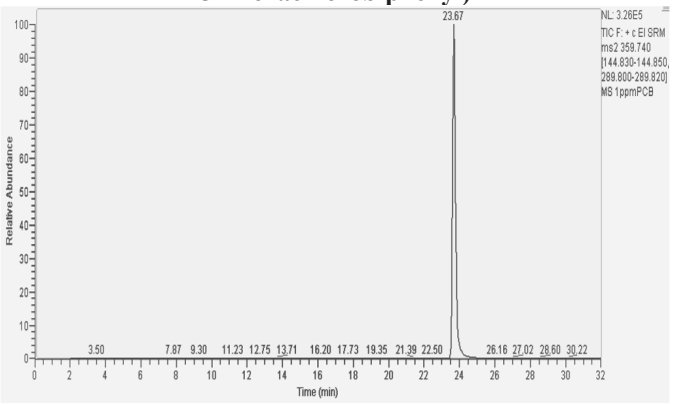

Fig. 4: Chromatogram showing PCB 169 in Puntius Intestine (10 Days)

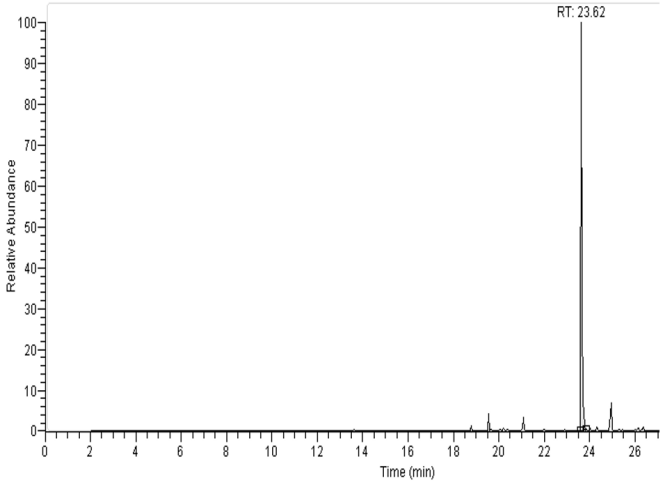

Fig. 5: Graph showing increasing Bioaccumulation of PCB 169

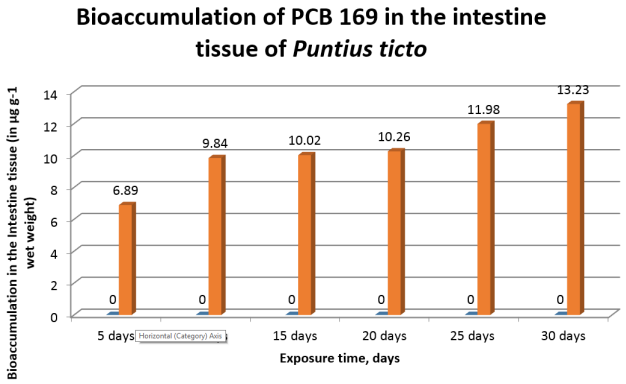

Fig. 6: T.S. Intestine of Puntius ticto exposed to PCB 169 for 10 days (Magnification: 40X)

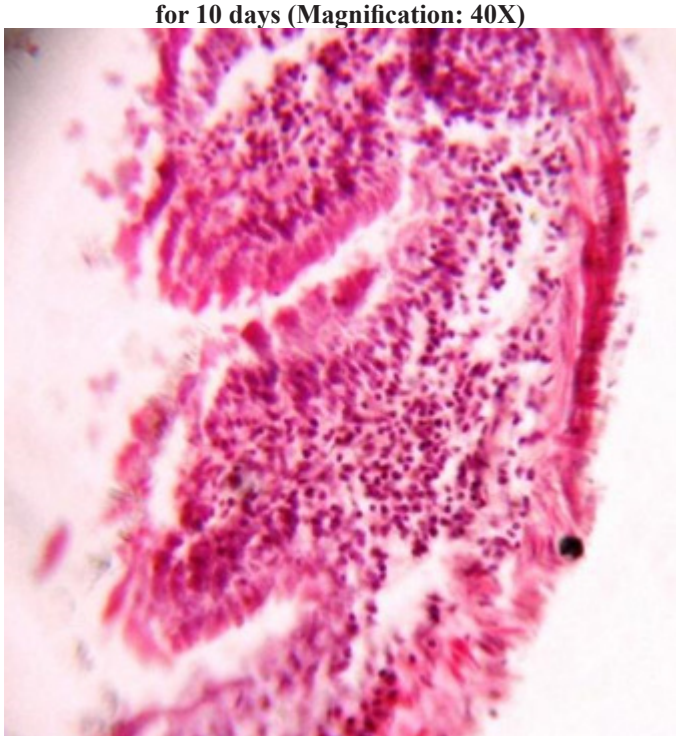

Fig. 7: T.S. Intestine of Puntius ticto exposed to PCB 169 for 20 days (Magnification: 40X)

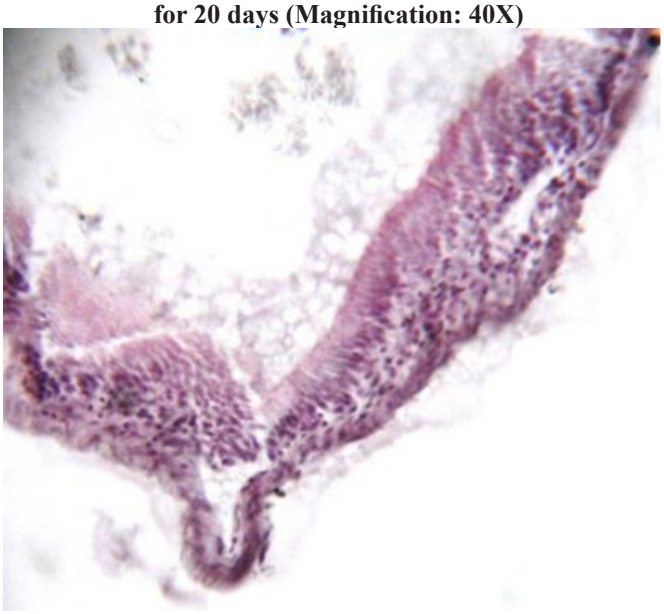

Fig. 8: T.S. Intestine of Puntius ticto exposed to PCB 169 for 30 days (Magnification: 40X)

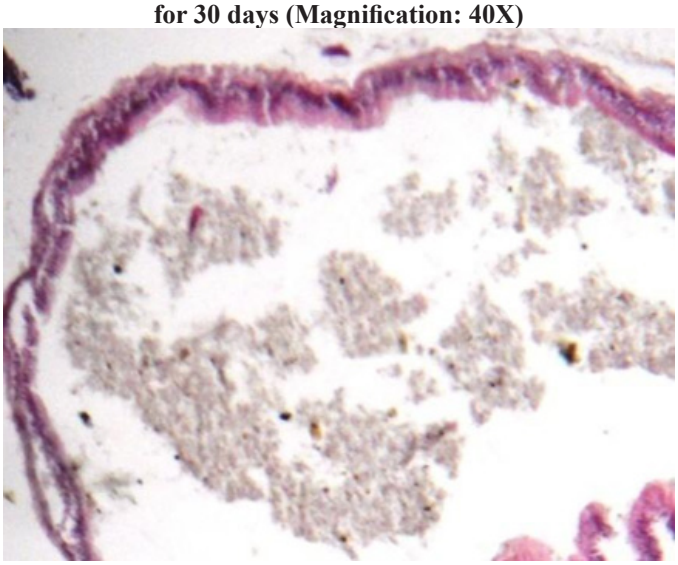

Table. 1: Characteristics of Dilution Water

Parameters	**Values ***
Temperature º C	25-27
pH	7.5-8.2
Total Alkalinity as $CaCO_3$	156-190
Total Hardness as $CaCO_3$	142-172
Ca Hardness as $CaCO_3$	80-94
Mg Hardness as $CaCO_3$	62-78
Dissolved Oxygen	6.9-7.3
Calcium as Ca	32-38
Magnesium as Mg	14-18
Sodium as Na	36-38
Potassium as K	2-4
Chloride	126

***All the values are expressed as mg/L except temperature and pH.**

Table 2: Bioaccumulation of PCB 169 in Puntius ticto.

Exposure time, days	Bioaccumulation in the Intestine tissue (in µg g^{-1} wet weight)
5	6.89
10	9.84
15	10.02
20	10.26
25	11.98
30	13.23

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